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N25C4A N25DX**

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EP 0864860 A **WO 1998/020974 A**
FR 002781886 A **US 6376233 A**
US 5643742 A

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NBPX**
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27/26 27/27 27/28 27/403 33/483 33/49 33/543**
Other: Online: **EPODOC, JAPIO, WPI**

(54) Abstract Title
A multiwell plate for electrochemical detection

(57) A multiwell plate (e.g. a microtiter plate) for electrochemical detection, comprises a plurality of wells 1 wherein at least one of the wells 1 has a sensing electrode 2 and a reference electrode 3 associated with it, a further counter electrode may also be associated. The electrodes may be made from screen printed gold or other noble metals. The electrodes may be positioned on the bottom of the wells (2, 3 figure 2a not shown). The electrodes may be interdigitated. The sensing electrode may be coated with an electro-conductive polymer (e.g. polypyrrolle, thiophene, furan or aniline). The sensing electrode may be further coated with a layer of bio-molecules or adaptor molecules (e.g. avidin or streptavidin) which are absorbed into the surface of the electrodes. The bio-molecules or adaptor molecules may be still further coated with cells. The electro-conductive cells may be doped with mobile or immobile anions (e.g. sulphate anions). The electro-conductive cells may be doped with anions capable of specific cation interactions (e.g. with calcium cations). The electro-conductive cells may be doped with anions capable of changing their redox state.

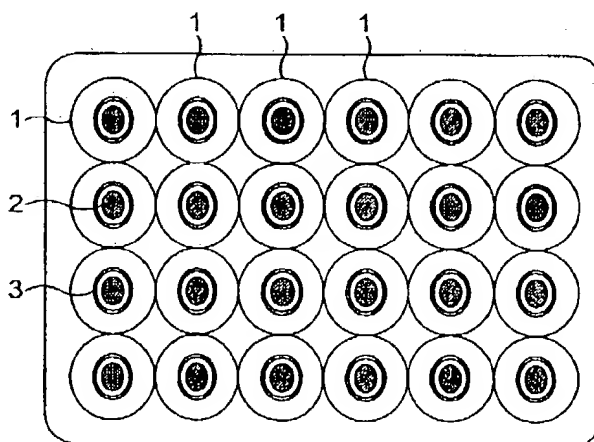


FIG. 1

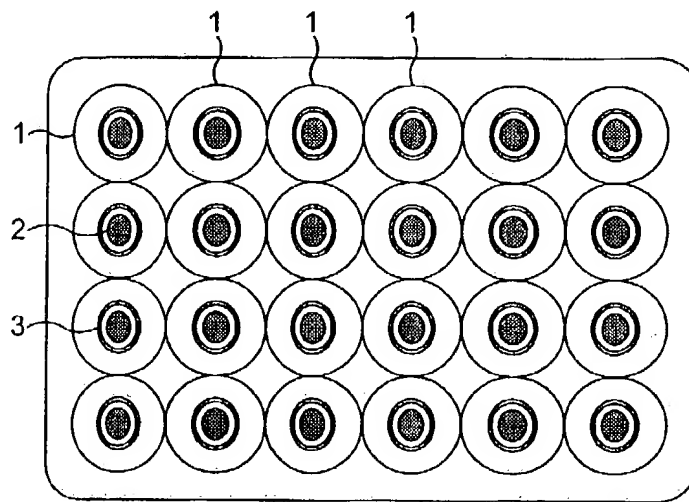


FIG. 1

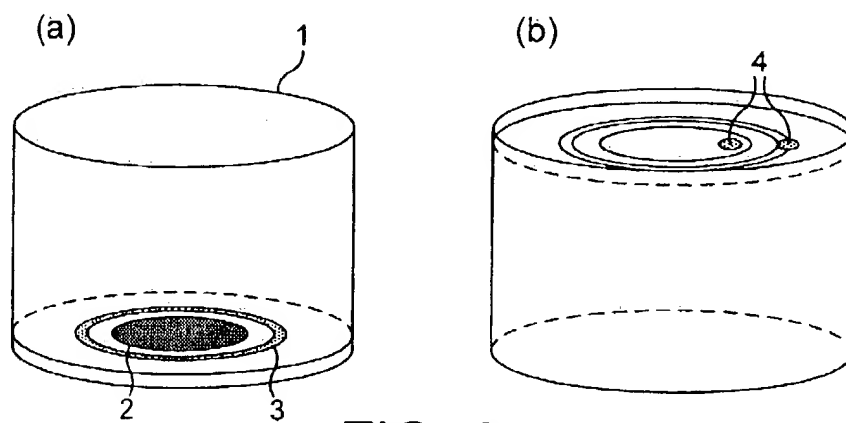
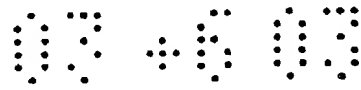
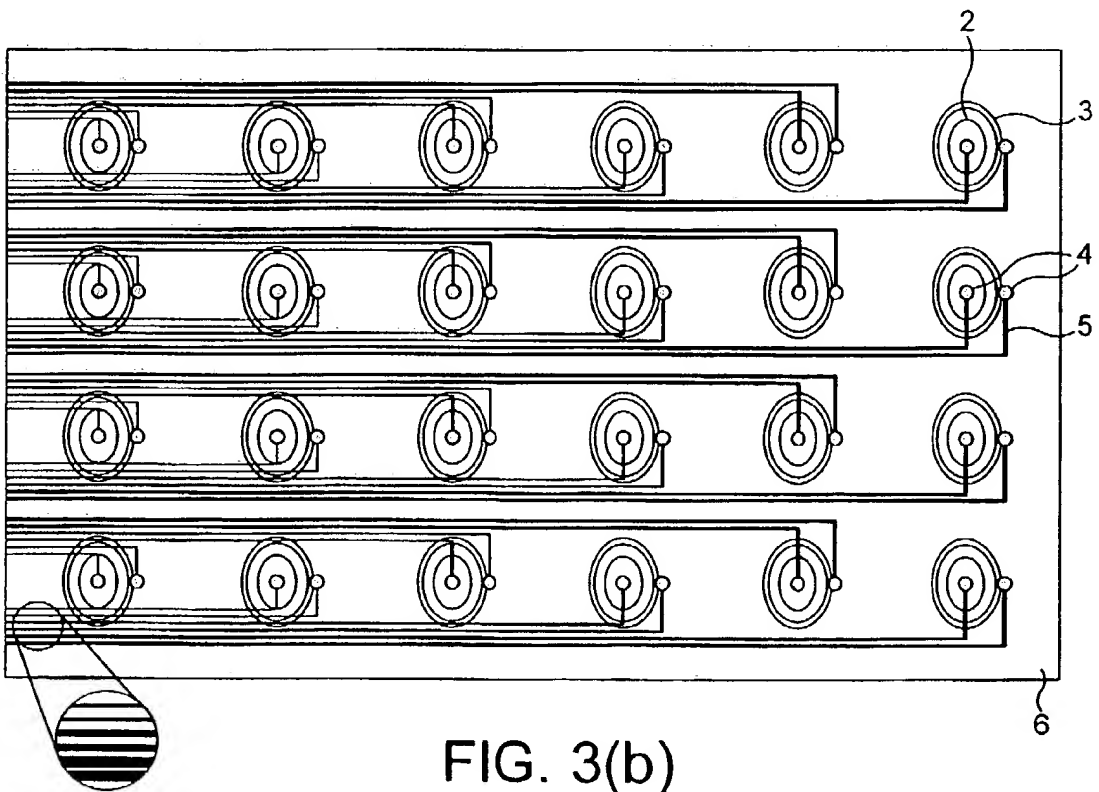
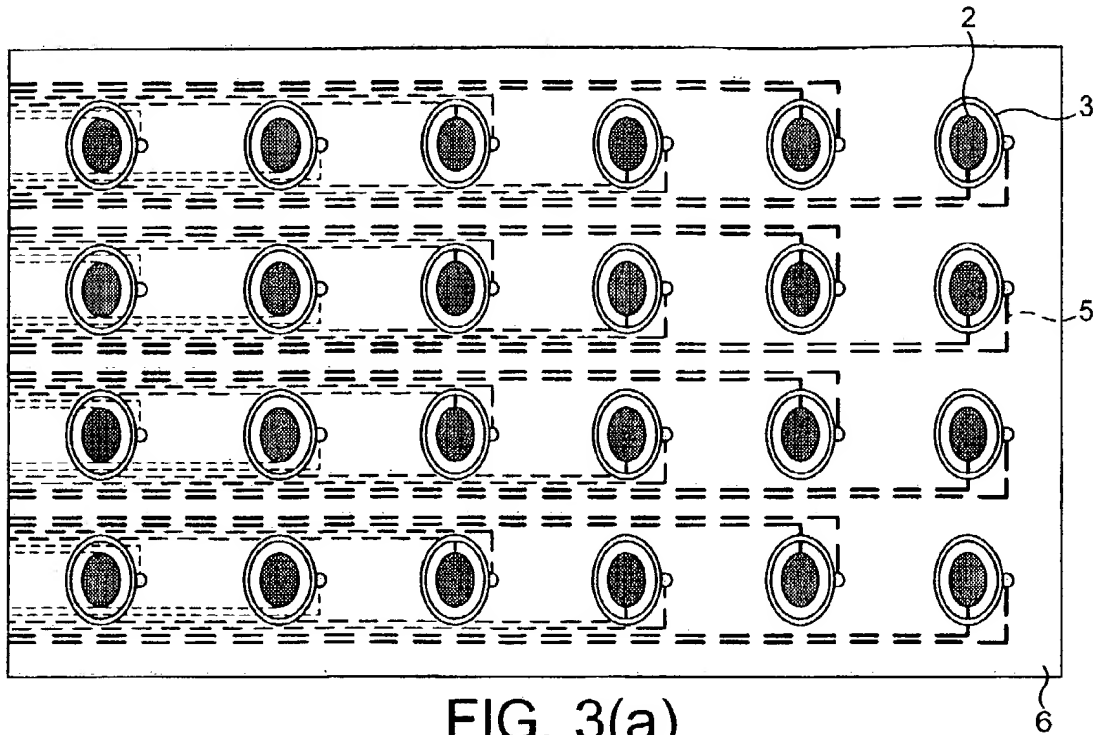


FIG. 2

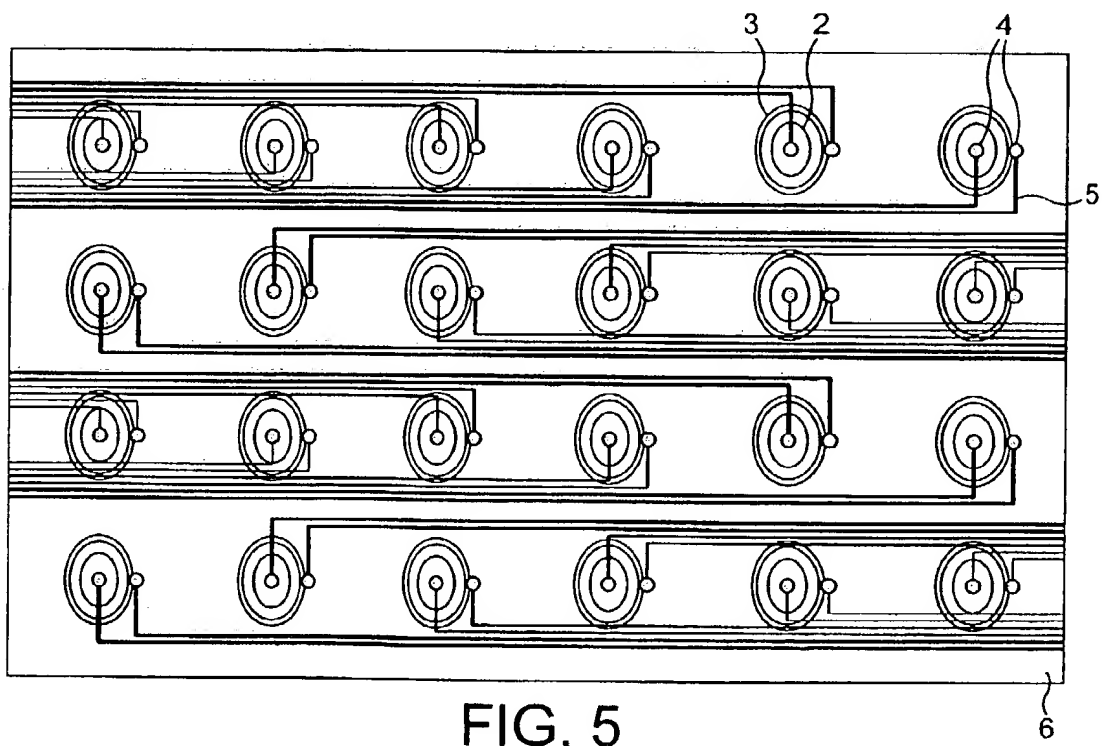
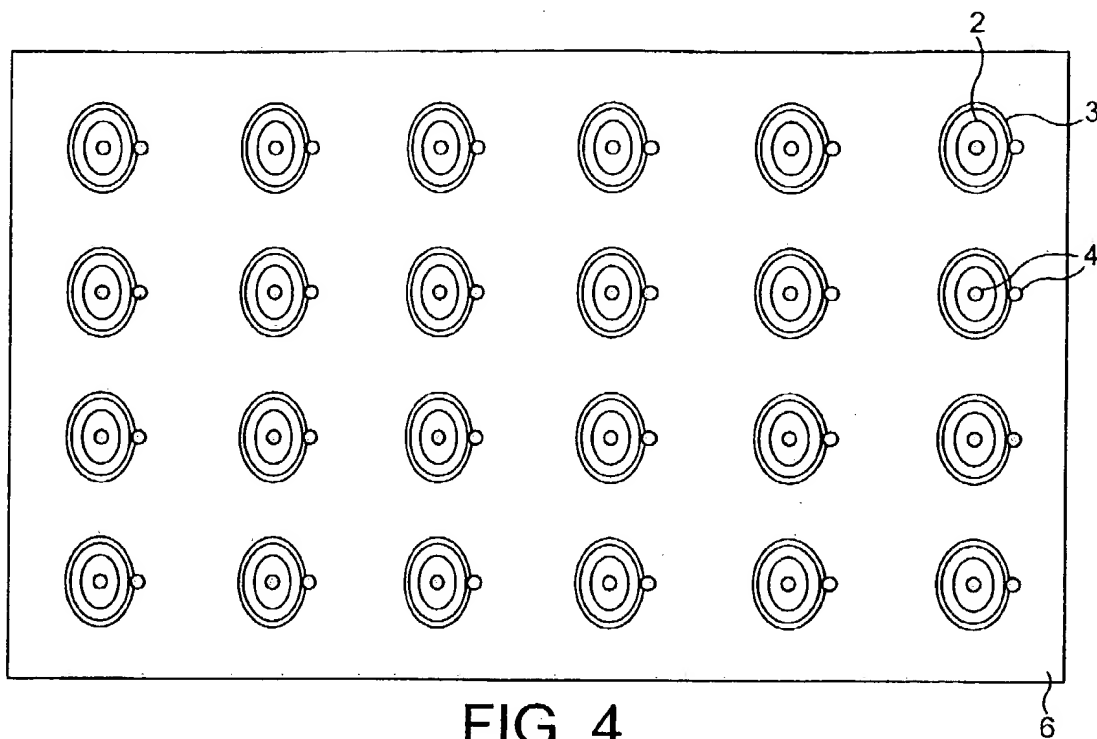


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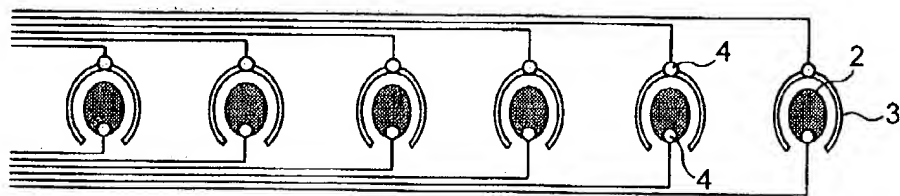


FIG. 6

Electrochemical Multi-well Plate

Field of the invention

The invention relates to multiwell plates
5 containing integrated electrochemical sensors for use
in methods of electrochemical analysis.

Background to the invention

Electrochemical analysis of ELISAs, cell
10 analysis, proteomics and other analytes by detection
of various local or internal changes in pH
(acidification), ionic strength or redox potential is
a promising and attractive method of instrument
analysis.

15

International application PCT/GB98/00548 (WO
98/37409) describes a potentiometric method of
electrochemical analysis using an electrochemical
sensing electrode comprising a metallic potentiometric
20 electrode coated with a layer of electroconductive
polymer containing immobilised bioreceptor molecules
which bind specifically to the analyte under test.
The presence of analyte is indicated by a change in
potential of the sensing electrode upon binding of
25 analyte to the immobilised bioreceptors, using an
ion-step detection procedure. This same sensor
configuration can also be employed at the bottom of
every well of a multiwell plate.

30

Description of the invention

In a first aspect the invention provides a multi-
well plate for use in methods of electrochemical
detection wherein at least one of the wells of the
multi-well plate has associated therewith a sensing
35 electrode and a reference electrode.

In a preferred embodiment each of the wells of

the multi-well plate will have an associated sensing electrode and reference electrode.

5 In a further aspect the invention provides A
method of electrochemical analysis of the response of
whole cells to a change in pH, ionic strength or
chemical composition of an electrolyte solution,
comprising the steps of:
10 (a) providing a multiwell plate according to the
invention wherein whole cells are attached to at least
one of the sensing electrodes;
(b) adding an electrolyte solution to the wells of the
multi-well plate thereby immersing the sensing and
reference electrodes in the electrolyte solution;
15 (c) monitoring the electric potential difference
between the sensing and reference electrodes when
immersed in the electrolyte solution;
(d) monitoring the electric potential difference
between the sensing and reference electrodes following
20 a change in the composition, pH or ionic strength of
the electrolyte solution, and thereby analysing the
response of the cells to the change in composition, pH
or ionic strength of the electrolyte solution.

25 An embodiment of the present invention will now
be described, by way of example only, with reference
to the accompanying Figures in which:

Figure 1 is a schematic representation of a section of
30 a multi-well plate according to the invention;

Figure 2(a) is an enlarged view of a single well of
the multi-well plate of Figure 1, Figure 2(b) is an
inverted view of the same well in which the underside
35 of the well is visible;

Figure 3(a) is a plan view of an array of sensing and

reference electrodes for incorporation into a multi-well plate according to the invention viewed from above, Figure 3(b) is a plan view of the underside of the array of sensing and reference electrodes shown in Figure 3(a);

Figure 4 is a plan view of a further array of sensing and reference electrodes for incorporation into a multi-well plate according to the invention viewed from above;

Figure 5 is a plan view of the underside of a further array of sensing and reference electrodes for use in a multi-well plate according to the invention;

Figure 6 is a plan view of a further array of sensing and reference electrodes for use in a multi-well plate according to the invention.

There is shown in Figure 1 a multi-well plate having a plurality of round wells 1. The plate will preferably be of similar size, configuration and construction to a standard microtiter plate. Most preferably the plate will be formed of an electrically insulating plastic material. Multi-well plates with various numbers of wells, for example 6, 12, 24 96, 384, 1536 and above, could be made.

Each of the wells has associated therewith a sensing electrode 2 and a reference electrode 3. In this embodiment the sensing electrode 2 is formed in a substantially circular spot positioned substantially centrally on the bottom of the well. The reference electrode is formed as a ring surrounding the sensing electrode. This configuration is selected for convenience for use with round wells and other arrangements can be envisaged. For example, it is not

essential for the reference electrode to be formed as a ring completely surrounding the sensing electrode (see Fig. 5).

5 Figure 2 shows an enlarged view of a single well of the multi-well plate. The sensing electrode and reference electrode are positioned on the bottom interior surface of the well (see Fig. 2(a)) such that they would be in contact with material (e.g.
10 electrolyte) added to the well. In other embodiments the electrodes could be positioned in the side walls of the wells. Electrical contact to the sensing and reference electrodes is provided via connections 4 through to the underside of the plate (shown in Fig.
15 2(b)).

A third electrode could also be associated with each pair of sensing and reference electrodes in order to allow for amperometric sensing.

20

Multi-well plates according to the invention may be manufactured in a variety of ways, using single or double-sided circuits. Arrays of reference and working electrodes may be assembled by screen
25 printing, etching and gold plating, or using thin film technologies. Conveniently, the array may be manufactured on a flat sheet or substrate, which may then form the bottom surface of the plate. Electrical connections to the sensing and reference electrodes
30 may be provided either on the same side of the substrate as the electrodes or through to the other side of the substrate (which will form the underside of the plate) for double sided circuits. The reference electrode-working electrode loop needs to be
35 kept as small as possible where required so no induction voltage is generated. If the circuit is single sided a ground plate can be added to the bottom

to further reduce any induction voltage.

Figure 3(a) shows an array of sensing and reference electrodes formed on a flat substrate 6 as a double-sided circuit. The sensing electrodes 2 and reference electrodes 3 are applied to one surface of the substrate (uppermost in Fig. 3(a)). Independent electrical connections 4 are provided from each of the sensing and reference electrodes through to the other side of the substrate. In this embodiment the connections 4 are protected and tracked to the edge of the plate via electrically conductive tracks 5 which run substantially parallel to each other. The enlarged insert in Fig. 3(b) illustrates how the widths of parallel conductive tracks may be varied in order to provide equal resistance to all points on the plate. The tracks can be connected to a single edge connection point/plug which enables the whole unit to be plugged into a detection instrument.

Figure 4 illustrates a further array of sensing and reference electrodes formed on a flat substrate 6 as a double-sided circuit. The sensing electrodes 2 and reference electrodes 3 are applied to one surface of the substrate (uppermost in Fig. 4). Independent electrical connections 4 are again provided from each of the sensing and reference electrodes through to the other side of the substrate. In this embodiment the connections 4 on the underside are designed to enable direct contact to a detection instrument, e.g. pin connectors.

For convenience the sensing electrode and reference electrodes can be linked to the measuring instrument by means of a special holder equipped with electrical contacts for connection of the sensing electrode and reference electrode and connected to the

measuring instrument by a cable or other means. A holder integral with the measuring instrument could also be used, making it possible to miniaturise the measuring system in terms of its overall dimensions.

5

Figure 5 illustrates a further array of sensing and reference electrodes formed on a flat substrate 6 as a double-sided circuit. The sensing electrodes 2 and reference electrodes 3 are applied to one surface of the substrate (below the plane of Figure 5). Independent electrical connections 4 are provided from each of the sensing and reference electrodes through to the other side of the substrate. In this embodiment the connections 4 are protected(?) and tracked to the edges of the plate via electrically conductive tracks 5.

Figure 6 shows a further array of sensing and reference electrodes which may be formed on a flat substrate as a single-sided circuit. In this embodiment the sensing electrode 2 is again formed as a substantially circular spot and the reference electrode is formed as an open ring around the sensing electrode. Independent connections 4 are provided to each of the sensing and reference electrodes on the same side of the substrate. The connections are tracked to the edge of the substrate via electrically conductive tracks 5, also formed on the same side of the substrate.

30

A substrate bearing an array of sensing and reference electrodes may be affixed to the bottom of an open tube array, with the side bearing the sensing and reference electrodes facing uppermost towards the open tube array, in order to form a multiwell plate. In this arrangement the uppermost surface of the substrate, bearing the array of sensing and reference

35

electrodes, forms the bottom interior surface of the wells. Most usually one pair of sensing and reference electrodes will be positioned in each well.

- 5 In a further embodiment each pair of sensing and reference electrodes may have associated therewith a third counter-electrode. The inclusion of a third electrode enables other forms of electrochemical analysis to be carried out, for example amperometry.
- 10 In one embodiment the third counter-electrode and sensing electrode may be formed as interdigitating electrodes, or as closely separated parallel lines in a variety of shapes. Such arrangements enable alternative forms of electrochemical analysis to be
- 15 carried out, for example amperometry, impedance, voltammetry, polarography, chronoamperometry, chronocoulometry and chronopotentiometry.

- 20 The sensing electrodes may be essentially any suitable electrode comprising a conductive or semi-conductive layer. Suitable electrodes include standard potentiometric electrodes possessing metallic or quasi-metallic conductivity which are stable in aqueous media, e.g. gold and other noble metal
- 25 electrodes. In a preferred embodiment the sensing electrode may comprise a plastic support or substrate with a conductive layer (preferably gold or other noble metals) electrochemically plated or directly screen-printed onto the plastic support. An adhesive
- 30 layer (e.g. carbon or copper) may be interposed between the plastic substrate and the conductive layer.

- 35 Sensing electrodes can be used without any further processing, or they can be electrochemically coated, preferably on at least a portion of at least one major surface, with a layer of electroconductive

polymer, e.g. polypyrrole.

The regular Ag/AgCl or calomel electrode can serve as a reference electrode.

5

In embodiments wherein one or more of the sensing electrodes are coated with an electroconductive polymer film as part of the sensing element, a thin film is deposited onto the surface of an electrically
10 conductive electrode by electrochemical synthesis from a monomer solution. The electrically conductive electrode is preferably a standard potentiometric electrode possessing metallic or quasi-metallic conductivity which is stable in aqueous media. As
15 will be illustrated in the examples included herein, electrodeposition of the electroconductive polymer film is carried out using a solution containing monomers, a polar solvent and a background electrolyte. Pyrrole is the preferred monomer, but
20 thiophene, furan or aniline could also be used. Combinations of two or more of these monomers may also be used, leading to the production of conductive copolymers.

25 The preferred supporting electrolyte is sodium dodecylsulphate but other electrolytes may be used. The electrolyte also serves as a doping agent. Deionised water is preferably used as the polar solvent.

30

The electrochemical polymerisation solution generally consists of an aqueous solution of monomers and supporting electrolyte. However, other components may be added to the polymerisation solution such as,
35 for example, components which provide specific functional groups which can be used as linkers for bioreceptors or for chemical modification of the

sensor surface (see WO 00/11473 and WO 98/37409).

Electrochemical polymerisation is typically carried out in a three-electrode cell comprising of sensor electrode(s) to be coated, the auxiliary electrode and the reference electrode. Suitable assemblies have been described in the prior art (see WO 00/11473 and references contained therein). Multiple sensor electrodes can be combined in a block with one electrical contact. An entire array of sensing electrodes may be coated in a single polymerisation reaction. This may use either a single auxiliary electrode or one auxiliary electrode per pair of sensing and reference electrodes. For example, arrays which include a third counter-electrode associated with each pair of sensing and reference electrodes (e.g. for amperometric analysis) may be coated using the third counter-electrode as the auxiliary electrode. In a further arrangement, the reference electrodes may be used as the auxiliary (counter) electrode for the polymerisation step. The reference electrode may be manipulated to function as an auxiliary electrode for polymerisation, for example with Ag/AgCl electrodes the ratio of Ag/AgCl may be temporarily altered such that it functions as an auxiliary electrode for polymerisation, and then restored to function as a reference electrode after polymerisation.

As is well known to persons skilled in the art, electroconductive polymers are often doped at the electrochemical synthesis stage in order to modify the structure and/or conduction properties of the polymer. As reported in a number of papers [4, 5], the ease with which ion exchange takes place and the rapidity with which ion equilibrium is attained for electroconductive polymers immersed in a solution are

essentially dependent on the size of the anti-ion introduced at the electrodeposition stage: the larger the ionic radius of the anti-ion, the more readily ion-exchange reactions take place and the more rapidly a state of equilibrium is reached. This is directly linked to the value and rate of change of the potential of the "metal electrode - electroconductive polymer" system in response to variation in the ion composition of the solution [6]. The type of the response (anionic, cationic, redox) and its rate can be determined during the polymerisation [5, 6].

A typical dopant anion is sulphate (SO_4^{2-}) which is incorporated during the polymerisation process, neutralising the positive charge on the polymer backbone. Sulphate is not readily released by ion exchange and thus helps to maintain the structure of the polymer.

It is possible to provide potentiometric sensitivity of the electroconductive polymer to one particular cation or anion. The ions of background electrolyte are immobile and able to react specifically with the ion of interest, e.g. calcium (cation), which specifically reacts with calcium and gives precipitated product (salt).

For redox and pH sensitive sensors it is preferred to use a salt whose anions have a large ionic radius as the background electrolyte when preparing the electrochemical polymerisation solution. In this case ion response is minimised and redox or pH response predominate, potentiometric response is provided by electron exchange between the polymer film and surrounding solution.

Suitable salts whose anions have large ionic

radius include sodium dodecyl sulphate and dextran sulphate. The concentration of these salts in the electrochemical polymerisation solution is varied according to the type of test within the range 0.0001
5 - 0.05 M.

Redox response can be increased by incorporating into the polymer dopant ions, which can change their redox state due to the changes in the surrounding
10 solution giving the sensor the additional change in redox state. The dopant should be in reduced form if one of the solution components is oxidized and vice versa. $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ can be given as an example for both cases. The concentration of these
15 electrolytes in the electrochemical polymerisation solution can be varied within the range 0.001 - 0.1 M to meet specific requirements of the test.

The surfaces of electroconductive polymer-coated
20 electrodes can be further modified by coating with biomolecules or other functional groups which can be used as linkers for bio molecules or for chemical modification of the sensor surface (see WO 00/11473, WO 98/37409 and WO 96/02001).

25 Biological molecules, for example bioreceptors, can be immobilised onto a sensor using well known techniques for solid phase coating. Biological molecules may be incorporated into the
30 electroconductive polymer during the polymerisation reaction, or they may be adsorbed onto the surface of the coated sensing electrode in a separate modification step after the polymer coating step, or they may be covalently linked to the polymer coating.

35 In a particular embodiment the biological molecules may be "adaptor molecules" which enable the

attachment of further molecules, or even whole cells
to the surface of the sensor via a specific binding
interaction. With the selection of appropriate
adaptor molecules it is also possible to manufacture
5 "universal" sensing electrodes containing adaptor
molecules capable of binding to a whole range of
different receptor molecules. Specificity for the
analyte under test is conferred on the "universal"
sensing electrode simply by binding to the adaptor
10 molecules receptors of the appropriate specificity.

The proteins avidin and streptavidin are
preferred for use as adaptor molecules.
Investigations carried out by the authors of the
15 declared invention have shown that avidin and
streptavidin immobilised in an electroconductive
polymer film, retain their native properties for an
extended period of time (at least one year and
possibly longer) and can be used throughout this
20 period to link with biotin conjugated receptors.
Techniques which allow the conjugation of biotin to a
wide range of different molecules are well known in
the art. Thus sensing electrodes with immobilised
avidin or streptavidin can easily made specific for a
25 given analyte merely by binding of the appropriate
biotinylated receptors via biotin/avidin or
biotin/streptavidin binding interactions.

Although avidin and streptavidin are the
30 preferred adaptor molecules it is within the scope of
the invention to use alternative adaptor molecules,
for example protein A, protein G, lectins and FITC.
The incorporation of adaptor molecules enables other
biomolecules or whole cells to be attached to the
35 surface of the sensing electrode, for example via
protein A/antibody, protein G/antibody, FITC/anti-FITC
or lectin/sugar binding interactions. Biomolecules or

whole cells may alternatively be absorbed directly or covalently bound to the sensor surface.

5 Multi-well plates according to the invention can be used in all areas and instruments currently designed for multi-well plates, especially fluidic handling robotics.

10 The electrochemical prepared multi-well plates can be used in the same manner as unmodified multi-well plates are used. For example, assays, cell analysis, drug discovery immobilisation protocols and fluidic regimes can all remain the same or very similar to that already used in standard multi-well or
15 microtiter plates. The only difference is in the mode of detection of result, being electrochemical (e.g. potentiometric, or amperometric if a three electrode array format is employed) with associated increased sensitivity and reduced time to result.

20 The multi-well plates of the invention are inexpensive to manufacture and so for convenience can be produced in a disposable format, intended to be used for a single electrochemical detection experiment
25 or a series of detection experiments and then thrown away.

Electrochemical multi-well plates according to the invention may be used in methods of
30 electrochemical analysis of analytes, such as, for example the methods described in the applicant's published International patent application WO 00/11473.

35 In a particularly useful application the multi-well plate can be used in the analysis of whole cells, for example in monitoring the response of whole cells

to analytes or to changes in conditions of pH, ionic strength or chemical composition of an electrolyte solution. In this embodiment whole cells may be adsorbed directly onto the surface of sensing electrodes coated with electroconductive polymer. Alternatively, whole cells may be attached to the sensing electrode indirectly via binding to a biomolecule immobilised in or adsorbed to the electroconductive polymer coating.

10

The multi-well plates of the invention may be advantageously used in the following applications: ELISA, cell analysis, drug discovery/toxicity, Ultra High-throughput screening, analysis of chemical and biological reactions, study of biological interactions, electrochemical assays of all descriptions, etc. This list is intended to be illustrative rather than limiting to the invention.

20

Claims:

1. A multi-well plate for use in methods of electrochemical detection wherein at least one of the wells of the multi-well plate has associated therewith a sensing electrode and a reference electrode.
2. A multi-well plate according to claim 1 wherein each of the wells of the multi-well plate has associated therewith a sensing electrode and a reference electrode.
3. A multi-well plate according to claim 1 or claim 2 wherein the sensing and reference electrodes are positioned on the bottom interior surface of the well(s).
4. A multi-well plate according to any one of claims 1 to 3 wherein the sensing electrode comprises an electroconductive electrode coated with a layer of electroconductive polymer.
5. A multi-well plate according to claim 4 wherein the sensing electrode is further coated with cells immobilised in, adsorbed to or attached to the layer of electroconductive polymer.
6. A multi-well plate according to claim 4 wherein the sensing electrode is further coated with biomolecules or adaptor molecules immobilised in, adsorbed to or attached to the layer of electroconductive polymer.
7. A multi-well plate according to claim 6 wherein the sensing electrode is coated with adaptor molecules immobilised in, adsorbed to or attached to the layer of electroconductive polymer, and still

further coated with cells attached to the adaptor molecules.

5 8. A multi-well plate according to any one of
claims 4 to 7 wherein the layer of electroconductive
polymer has been doped with mobile anions of large
ionic radius.

10 9. A multi-well plate according to any one of
claims 4 to 7 wherein the layer of electroconductive
polymer has been doped with anions which are immobile
in the electroconductive polymer.

15 10. A multi-well plate according to any one of
claims 4 to 7 wherein the layer of electroconductive
polymer has been doped with anions carrying a large
amount of negative charge.

20 11. A multi-well plate according to any one of
claims 4 to 7 wherein the layer of electroconductive
polymer has been doped with anions capable of specific
interaction with cations.

25 12. A multi-well plate according to any one of
claims 4 to 7 wherein the layer of electroconductive
polymer has been doped with anions capable of changing
their redox state.

30 13. A multi-well plate according to any one of
claims 1 to 12 which further comprises a third counter
electrode associated with each of the sensing and
reference electrodes.

35 14. A multi-well plate according to any one of
claims 1 to 12 which further comprises at least one
pair of interdigitating electrodes.



Application No: GB 0207114.0
Claims searched: 1-14

Examiner: Peter Mason
Date of search: 17 October 2002

Patents Act 1977 Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK CI (Ed.T): G1N: (NBPX, NBPC, NBAC, NBAA, NBPE)

Int CI (Ed.7): B01J: 19/00
G01N: 27/06, 27/07, 27/26, 27/27, 27/28, 27/403, 33/487, 33/483, 33/493, 33/543
C12M: 1/00, 1/18,

Other: Online: EPODOC, JAPIO, WPI

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
A	EP 0,864,860 A1 (SHIMADZU CORPORATION) See whole document, note electrode configuration (104) as shown in figure 1A.	-
X	FR 2,781,886 A (COMMISSARIAT ENERGIE ATOMIQUE) See EPODOC and WPI abstracts, also see for example figure 1G.	1-7
X,&	WO 98/20974 A1 (DEUTSCHE ITT INDUSTRIES GMBH)	1-3, & 14
X,E,&	US 6,376,233 B1 (MICRONAS INTERNATIONAL GMBH) See whole document, note particularly references to interdigitated sensors in column 2, line 44 - 53.	1-3, & 14
A	US 5,643,742 (CELLSTAT TECHNOLOGIES INC) See whole document, note electrodes associated with the lid (10), of the multi well plate as shown in figure 1.	-

X Document indicating lack of novelty or inventive step
Y Document indicating lack of inventive step if combined with one or more other documents of same category.

& Member of the same patent family

A Document indicating technological background and/or state of the art.
P Document published on or after the declared priority date but before the filing date of this invention.
E Patent document published on or after, but with priority date earlier than, the filing date of this application.